PATENT
Customer No. 22,852
Attorney Docket No. 03495.0151-02

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	
	)	
Sanchis et al.	)	Group Art Unit: not yet assigned
	)	
Serial No.: not yet assigned (Divisional of Serial No. 09/037,629)	)	Examiner: not yet assigned
	)	
Filed: concurrently herewith	)	
	)	
For: NUCLEOTIDE SEQUENCES CODING FOR POLYPEPTIDES ENDOWED WITH A LARVICIDAL ACTIVITY TOWARD LEPIDOPTERA	)	

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

PRELIMINARY AMENDMENT

Prior to the examination of the above application, please amend this application as follows:

IN THE CLAIMS:

Please cancel claims 1-14, 19-20, 28, and 32-36; amend claims 15-18, 21-27, and 29-31; and add claims 37 to 41 as set forth below:

15. A recombinant expression and cloning vector containing a nucleotide sequence coding for at least part of the N-terminal region of a polypeptide specifically toxic toward larvae of Lepidoptera of the family Noctuidae.

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16. The recombinant expression and cloning vector according to Claim 15, comprising a *Hind*III-*Pst*I DNA fragment constituted uniquely of DNA derived from the *aizawai* 7-29 strain.

17. A modified bacterial strain comprising a nucleotide sequence coding for at least part of the N-terminal region of a polypeptide specifically toxic toward larvae of Lepidoptera of the family Noctuidae.

18. The bacterial strain according to Claim 17, comprising at least one recombinant vector according to Claim 15 or 16.

21. A process for obtaining a nucleotide sequence coding for at least a part of the N-terminal region of a polypeptide toxic specifically toward Lepidoptera of the family Noctuidae comprising the following steps:

(a) carrying out a hybridization between a sequence of nucleotides from a strain of *B. thuringiensis* active against *S. litteralis*, and one or more sequences of nucleotides utilized as hybridization probes derived from

(i) the 5' part of a restriction fragment of a gene for the δ-endotoxin of *B. thuringiensis* that codes for the N-terminal part of a polypeptide toxic toward Lepidoptera, or

(ii) the 3' part of a restriction fragment of a gene for the δ-endotoxin of *B. thuringiensis* coding for the COOH part of a polypeptide toxic toward Lepidoptera,

(b) isolating the fragment,

(c) cloning the fragment in a vector, followed by its purification.

22. The process according to Claim 21, wherein the hybridization probes utilized are obtained from a gene for a δ-endotoxin derived from a *aizawai* 7-29 strain

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coding for a protein of 130 kDa active against *P. brassicae* and inactive toward *S. littoralis*.

23. The process according to Claim 21 or 22, wherein the fragment recombined with the vector in the cloning step is elaborated from at least one sequence of nucleotides derived from at least one recombinant vector containing a sequence of nucleotides from at least one strain of *B. thuringiensis*.

24. The process according to Claim 23, wherein the fragment recombined with the vector in the cloning step is elaborated from several sequences of nucleotides from at least 2 different strains of *B. thuringiensis* possessing the same restriction maps and containing all or part of the sequences of nucleotides capable of coding for a polypeptide active toward *S. littoralis*.

25. The process according to Claim 23, wherein the fragment recombined with the vector in the cloning step is elaborated from a *Hind*III-*Pst*I restriction fragment derived from the *aizawai* 7-29 strain.

26. The process according to Claim 24, wherein the fragment recombined with the vector in the cloning step is elaborated from a *Hind*III-*Hinc*II restriction fragment derived from the *entomocidus* 6-01 strain and from a *Hind*III-*Pst*I restriction fragment derived from the *aizawai* 7-29 strain.

27. The process according to Claim 22, wherein the fragment recombined according to Claim 25 is carried by a plasmid pHTA6 and the restriction fragments recombined according to Claim 26, *Hind*III-*Hinc*II and *Hind*III-*Hinc*II are carried by the respective recombinant plasmids pHTE6 and pHTA6, said plasmids pHTE6 and pHTA6 being isolated with the aid of a probe constituted by a *Pvu*II fragment of 2 kb of the

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plasmid pBT15-88 corresponding to the internal part of a gene for the chromosomal crystal of the *Berliner* 1715 strain, from transforming clones containing nucleotide sequences derived from *B. thuringiensis* strains active toward larvae of Lepidoptera.

29. A process for producing a polypeptide toxic towards Lepidoptera comprising the steps of:

(a) expressing the polypeptide in a microorganism capable of expressing recombinant vectors according to any one of claims 15, 16, 37, or 38; and

(b) collecting the expressed polypeptide.

30. The process according to Claim 29, wherein the recombinant vectors are introduced into microorganisms living in the environment or in association with plants.

31. The process according to Claim 29 or 30, wherein the recombinant vectors are introduced into microorganisms in combination with different δ-endotoxin genes.

37. A recombinant expression and cloning vector according to Claim 15, wherein said nucleotide sequence is capable of hybridizing with a gene that expresses a polypeptide having the amino acid sequence of SEQ ID NO: 2 or larvicidal fragments thereof.

38. A recombinant expression and cloning vector according to Claim 15, wherein the encoded polypeptide is capable of forming an immunological complex with antibodies directed against a polypeptide having the animo acid sequence of SEQ ID NO: 2 or larvicidal fragments thereof.

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39. A modified bacterial strain according to Claim 17, wherein said nucleotide sequence is capable of hybridizing with a gene that expresses a polypeptide having the amino acid sequence of SEQ ID NO: 2 or larvicidal fragments thereof.

40. The process according to Claim 29, wherein the microorganism is selected from the group consisting of *E. coli*, *B. subtilis, B. cereus, or B. thuringiensis.*

41. A process for producing plants resistant to *S. littoralis* comprising the steps of transforming a plant sensitive to *S. littoralis* with a recombinant vector according to any one of claims 15, 16, 37, or 38, wherein the transformed plant is capable of a polypeptide toxic toward *S. littoralis.*

REMARKS

Applicants have amended the claims to correct improper multiple dependencies and to place them in better format for examination. New claims 37-41 are supported by the application and original claims as filed. No new matter is added by this amendment.

If there is any fee due in connection with the filing of this Preliminary Amendment, please charge the fee to our Deposit Account No. 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER, L.L.P.

Dated: August 1, 2001

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APPENDIX OF AMENDED CLAIMS

1. (Amended) A recombinant expression and cloning vector containing a [at least part of the] nucleotide sequence coding for at least part of the N-terminal region of a polypeptide specifically toxic toward larvae of Lepidoptera of the family Noctuidae [such as defined in any one of the Claims 1 to 14].

16. (Amended) The recombinant expression and cloning vector [Plasmid] according to Claim 15, [characterized in that it is pHT671 as represented in figure 4, or pHT71] comprising a *Hind*III-*Pst*I DNA fragment constituted uniquely of DNA derived from the *aizawai* 7-29 strain.

17. (Amended) A modified bacterial strain comprising a nucleotide sequence coding for at least part of the N-terminal region of a polypeptide specifically toxic toward larvae of Lepidoptera of the family Noctuidae [strains, characterized in that after transformation they contain a sequence of nucleotides according to one of the Claims 1 to 14].

18. (Amended) The bacterial strain according to Claim 17, comprising [characterized in that it contains] at least one recombinant vector according to Claim 15 or 16.

21. (Amended) A process [procedure] for obtaining a nucleotide sequence coding for at least a part of the N-terminal region of a polypeptide toxic specifically toward [towards] Lepidoptera of the family [of the] Noctuidae comprising [,and preferentially towards S. littoralis, characterized by] the following steps:

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(a) [the] carrying out [of] a hybridization between a sequence of nucleotides from a strain of *B. thuringiensis* active against *S. litteralis*, [on the one hand] and [,on the other,] one or more [several] sequences of nucleotides utilized as hybridization probes derived from

(i) the 5' part of a restriction fragment of a gene for the δ-endotoxin of *B. thuringiensis* that codes [,this part coding] for the N-terminal part of a polypeptide toxic toward [towards the] Lepidoptera, or [and derived from]

(ii) the 3' part of a restriction fragment of a gene for the δ-endotoxin of *B. thuringiensis* [this fragment] coding for the COOH part of a polypeptide toxic toward Lepidoptera [the polypeptide],

(b) isolating [the isolation of] the fragment,

(c) cloning the fragment [its cloning] in a vector, followed by its purification.

22. (Amended) The process [procedure] according to Claim 21, wherein [characterized in that] the hybridization probes utilized are obtained from a gene for a δ-endotoxin derived from a *aizawai* 7-29 strain coding for a protein of 130 kDa active against *P. brassicae* and inactive toward [towards] *S. littoralis*[, this gene having been cloned in the recombinant plasmid pHTA2].

23. (Amended) The process [procedure] according to Claim 21 or 22, wherein [characterized in that] the fragment recombined with the vector in the cloning step is elaborated from at least one sequence of nucleotides derived from at least one recombinant vector containing a sequence of nucleotides from at least one strain of *B. thuringiensis*.

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24. (Amended) The process [procedure] according to Claim 23, wherein [characterized in that] the fragment recombined with the vector in the cloning step is elaborated from several sequences of nucleotides from at least 2 different strains of *B. thuringiensis*[,] possessing the same restriction maps and [themselves] containing all or part of the sequences of nucleotides capable of coding for a polypeptide active toward [preferentially towards] *S. littoralis*.

25. (Amended) The process [procedure] according to Claim 23, wherein [characterized in that] the fragment recombined with the vector in the cloning step is elaborated from a *Hind*III-*Pst*I restriction fragment derived from the *aizawai* 7-29 strain.

26. (Amended) The process [procedure] according to Claim 24, wherein [characterized in that] the fragment recombined with the vector in the cloning step is elaborated from a *Hind*III-*Hinc*II restriction fragment derived from the *entomocidus* 6-01 strain and from a *Hind*III-*Pst*I restriction fragment derived from the *aizawai* 7-29 strain.

27. (Amended) The process [procedure] according to Claim 22, wherein [characterized in that] the fragment recombined according to Claim 25 is carried [preferentially] by a plasmid pHTA6 and the restriction fragments recombined according to Claim 26, *Hind*III-*Hinc*II and *Hind*III-*Hinc*II are carried [preferentially] by the respective recombinant plasmids pHTE6 and pHTA6, [the] said plasmids pHTE6 and pHTA6 being isolated with the aid of a probe constituted by a *Pvu*II fragment of 2 kb of the plasmid pBT15-88 corresponding to the internal part of a gene for the chromosomal crystal of the *Berliner* 1715 strain, from transforming clones containing nucleotide sequences derived from *B. thuringiensis* strains active toward [towards] larvae of [the] Lepidoptera[, inter-alia S. littoralis].

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29. (Amended) A process for producing [Application of the nucleotide sequences according to any one of the Claims 1 to 14 to produce] a polypeptide toxic towards Lepidoptera[, and preferentially S. littoralis, in microorganisms] comprising the steps of:

(a) expressing the polypeptide in a microorganism capable of expressing recombinant vectors according to any one of claims 15, 16, 37, or 38; and

(b) collecting the expressed polypeptide [containing these sequences such as E. coli, B. subtilis, B. cereus, or B. thuringiensis].

30. (Amended) The process [Application] according to Claim 29, wherein the recombinant vectors [characterized in that the sequences of nucleotides] are introduced into microorganisms living in the environment or in association with plants [such as Pseudomonas, Azospirillum or Rhizobium and capable of expressing recombinant vectors containing these sequences].

31. (Amended) The process [Application] according to Claim 29 or 30, wherein the recombinant vectors [characterized in that the nucleotide sequences] are introduced into microorganisms in combination with different δ-endotoxin genes.

37. (New) A recombinant expression and cloning vector according to Claim 15, wherein said nucleotide sequence is capable of hybridizing with a gene that expresses a polypeptide having the amino acid sequence of SEQ ID NO: 2 or larvicidal fragments thereof.

38. (New) A recombinant expression and cloning vector according to Claim 15, wherein the encoded polypeptide is capable of forming an immunological complex with

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antibodies directed against a polypeptide having the animo acid sequence of SEQ ID NO: 2 or larvicidal fragments thereof.

39. (New) A modified bacterial strain according to Claim 17, wherein said nucleotide sequence is capable of hybridizing with a gene that expresses a polypeptide having the amino acid sequence of SEQ ID NO: 2 or larvicidal fragments thereof.

40. (New) The process according to Claim 29, wherein the microorganism is selected from the group consisting of *E. coli*, *B. subtilis, B. cereus, or B. thuringiensis.*

41. (New) A process for producing plants resistant to *S. littoralis* comprising the steps of transforming a plant sensitive to *S. littoralis* with a recombinant vector according to any one of claims 15, 16, 37, or 38, wherein the transformed plant is capable of a polypeptide toxic toward *S. littoralis*.

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